THE RELATION BETWEEN OXIDASE AND CATALASE IN PLANT TISSUES

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(WITH ONE FIGURE)

In a previous paper[1] the writer has pointed out that although substances which act as oxidases or peroxidases usually decompose hydrogen peroxide, and although many authors think that there is a causal connection between the two processes, yet in the case of colloidal platinum they are quite independent. The question then arises, to what extent does this apply to the living cell? This is of particular interest in view of the fact that some investigators suppose that oxidase action depends in some way upon the activity of a catalase.

A few instances are on record in which, by some special treatment, plant oxidases have been prepared so as to give no catalase action. LIEBERMANN[2] found that by shaking an extract of malt with mercuric oxide and magnesia, or by heating the extract to 80° C., its catalase activity was destroyed but it still exhibited some peroxidase action. LOEW[3] reports that after treating an aqueous extract of fresh tobacco with one-fifth its volume of absolute alcohol it had no action on hydrogen peroxide, but still activated directly the oxidation of gum guaiac. Finally, KASANSKI[4] has shown that the catalase action of certain plant and animal extracts may be destroyed by the addition of strong solutions of pyrogallol or sugar, without completely inhibiting their peroxidase action.

More conclusive evidence that peroxidase action is independent of any ability to decompose hydrogen peroxide was obtained by the writer from a study of pineapple extracts. Pineapple juice always contains very active peroxidases; catalase reactions were

[1] REED, G. B., BOT. GAZ. **62**:233–238. 1916.

[2] LIEBERMANN, P., and L., Pflüger's Archiv. **108**:489–495. 1905.

[3] LOEW, O., Report no. 68. U.S. Dept. Agric., p. 47. 1901.

[4] KASANSKI, Biochem. Zeit. **39**:64–72. 1911.

only obtained, however, under certain conditions which are concerned with the stage of development of the fruit.

After some preliminary experimentation, 3 fruits of the same variety were selected. One was quite ripe and soft; another, although it had turned yellow, was still rather hard; the third had only partly lost its green color. The juice of each of these fruits was pressed out separately in a mortar, filtered, and the catalase activity determined by mixing 2 cc. of the juice in each case with 100 cc. of 0.05 M hydrogen peroxide and measuring the pressure of the oxygen evolved. For this purpose the hydrogen peroxide was placed in a 300 cc. bottle which was provided with a 3-hole stopper carrying a small separatory funnel in which the juice was placed, a paraffin manometer and a tube, closed by a stopcock, for the equalization of the initial pressure. The whole apparatus was submerged in a tank of water with a glass wall, the temperature of which was kept constant. After all the solutions had reached the same temperature the stopcock of the funnel was open and the juice allowed to run into the peroxide. On closing the stopcock of the funnel, as well as that of the pressure tube which had previously been open, the reaction was started

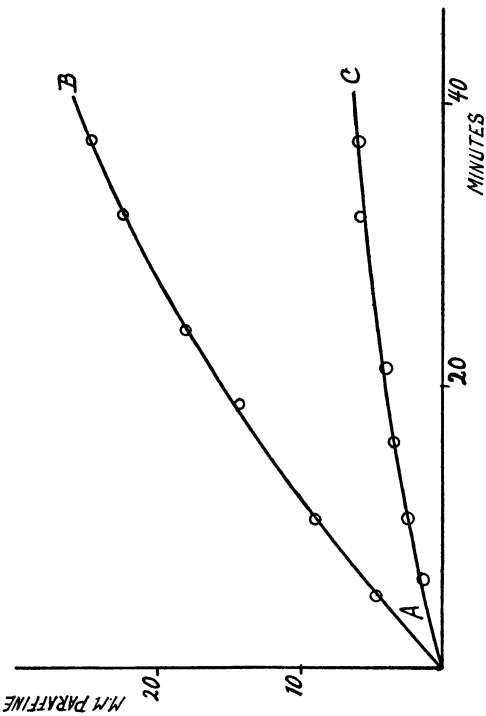

FIG. 1.—Curves representing decomposition of hydrogen peroxide: upper curve AB represents action of an extract of a well ripened pineapple; lower curve AC represents action of an extract of a partially green pineapple; ordinates represent pressure of oxygen produced (expressed in mm. of liquid paraffin in a paraffin manometer); abscissae represent time in minutes.

at atmospheric pressure.[5] The glass wall of the tank allowed easy reading of the manometer.

The curve AB (fig. 1) shows the rate of decomposition of the peroxide (as shown by the manometer readings) with the juice of the ripe fruit. Comparing this with AC of the same figure, which represents the rate of decomposition by the juice of the partially ripe fruit, it will be apparent that the latter has much less catalase activity than the former. Moreover, the juice of the green fruit showed no action on this concentration of hydrogen peroxide, or on a stronger solution, whether measured by the pressure method or by simply observing the evolution of bubbles of gas. As might be expected from these results, different fruits exhibit great variation in their catalase activity, but in every case the juice of those in a more or less green condition showed no action in decomposing hydrogen peroxide.

All of these fruits, however, showed approximately the same peroxidase activity, as the following determinations indicate. From each kind of fruit 10 cc. of juice was mixed separately with 100 cc. of 2 per cent pyrogallol solution containing 0.05 M hydrogen peroxide, placed in open beakers and maintained at 18° C. for two hours. The purpurogallin formed from the oxidation was then filtered off, dried, and weighed, after the method of BACH and CHODAT.

Comparing these results, stated in table I, with the previous measurements on the rate of hydrogen peroxide decomposition by

TABLE I

AMOUNT OF PURPUROGALLIN FORMED IN OXIDATION OF 100 CC. OF 2 PER CENT PYROGALLOL CONTAINING 0.05 M HYDROGEN PEROXIDE AND 10 CC. OF EXTRACT

Peroxidase from	Gm. purpurogallin formed in 2 hours
Ripe pineapple	0.986
Partially ripe pineapple	1.020
Green pineapple	1.096
Control; boiled pineapple	0.081

[5] The simpler method of titrating the unused hydrogen peroxide with permanganate could not be satisfactorily employed under these conditions, since the extract of the ferment had a reducing action on the permanganate.

the same extracts, it is evident that the peroxidase activity of pineapple juice is not dependent upon the rate of decomposition of hydrogen peroxide.

From the writer's studies on the behavior of platinum black (*loc. cit.*), in which it was shown that factors which influenced the catalase action have no effect on the oxidative activity, and from the fact that in certain stages of their development pineapples contain oxidases but no catalase, we may conclude that the substances which effect the decomposition of hydrogen peroxide are not of necessity concerned with the enzymes which accelerate peroxide oxidations. It may be added that the fact that catalase is not universally present in living cells, as LOEW and others suppose, has considerable theoretical interest.

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